Depuration of copper nitrate and chloride in the indian brown mussel *Perna indica*

Élimination du nitrate de cuivre et du chlorure de cuivre chez la moule indienne Perna indica

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Abstract

It is understood that the rate of bioaccumulation and subsequent depuration of heavy metals in marine animals is influenced by the chemical state, as well as the kinetics of transformation between different species of chemical salts dissolved in water. The Indian Brown mussel, *Perna indica*, was exposed to copper nitrate and chloride, individually, to analyse the rate of accumulation, depuration, oxygen uptake and filtration rates. When supplied in very low quantities, environmentally realistic concentrations, the rate of accumulation of copper nitrate and chlorides showed variations. When the animals were transferred to sea water, these two salts did not show any clear cut differences in the depuration rate. Irrespective of the previous accumulation history, the quantity of metal thrown out was always more when the body burden was high. This indicates that irrespective of the salt form of metals, the rate of depuration was controlled by the quantity of copper rather than salt forms supplied. Further, animals exposed to copper nitrate consumed lesser quantity of oxygen, compared to those exposed to copper chloride, although the load in the whole tissue was low (during accumulation). However, the rate of filtration decreased as a function of time of exposure to these salts and during depuration, between periods, the rate of filtration by those animals exposed to copper chloride was significant.

Résumé

Il est admis que la vitesse de bioaccumulation et d'élimination ultérieure des métaux lourds chez les organismes marins est influencée par l'état chimique ainsi que par la cinétique de transformation entre différentes espèces de sels chimiques en solution dans l'eau. La moule indienne *Perna indica* a été exposée séparément à du nitrate de cuivre et à du chlorure de cuivre afin d'analyser les vitesses d'accumulation et d'élimination, l'absorption d'oxygène et les taux de filtration. Avec des apports en très faible quantité et à des concentrations plausibles dans le milieu naturel, les taux d'accumulation du nitrate et du chlorure de cuivre présentent des variations. Après transfert des moules en eau de mer, on n'observe pas de différence très nette entre les temps d'élimination des deux sels. Quel que soit l'historique d'accumulation préalable, la quantité de métal relarguée est toujours supérieure lorsque la charge en métal dans l'organisme est plus élevée. Ceci indique que, quelle que soit la forme sous laquelle se présentent les sels, la vitesse de relargage est régie par la quantité de cuivre plutôt que par la forme des sels. Par ailleurs, les moules exposées au nitrate de cuivre consomment moins d'oxygène que celles exposées au chlorure de cuivre, bien que la charge dans l'ensemble des tissus soit faible (pendant l'accumulation). Néanmoins, le taux de filtration diminue en fonction de la durée d'exposition à ces sels, et au cours de la phase de purification entre ces périodes, le taux de filtration des moules exposées au chlorure de cuivre est important.

INTRODUCTION

Of late, heavy metal pollution of the aquatic environment has received considerable attention all over the world. These trace metals are toxic at low levels and get accumulated into the animal tissues easily. However, the mechanism by which these trace metals are concentrated in organisms is still not well understood. The chemical state, as well as the kinetics of transformation between different species of dissolved trace metals in sea water, are important to elucidate the bioaccumulation of heavy metals. Further, biological availability of a metal in the water is related to its chemical form and can profoundly affect its uptake, storage and toxicological consequences. Not much information is available on adverse effects of different salt forms of a particular heavy metal and their influence on metabolic activities of aquatic organisms. The importance of the influence of the available chemical forms of the metal, in the sea water in determining bioaccumulation of heavy metals has been emphasised by Allen *et al.* (1980), Martincic *et al.* (1984), Mohan *et al.* (1986) and Prabhudeva and Menon (1988).

The results presented in this paper report effects of nitrate and chloride forms of copper on accumulation, oxygen consumption, filtration and depuration in the indian brown mussel *Perna indica*, which could be an important candidate in the "Mussel Watch" programme.

Material and methods

Perna indica (20-25 mm shell length) collected from the Shakthikulangara rocky beach (8° 56'N; 76° 35'E) were kept in raw sea water (32 ± 1 °C) for 48 h prior to the experiments. Raw sea water collected from unpolluted area off Cochin was stabilised, sanitised and filtered by passing through a bio-filter and then used for the experiments.

Analar grade copper nitrate and copper chloride were the source of copper. 96 mussels were exposed in polythene tubs of 500 litres capacity containing 400 litres of the toxicant solution. Quadruplicate experiments were conducted per toxicant and control. The duration of the experiment was 16 days in case of accumulation and 7 days in case of depuration. The test solution was replenished every 24 h and the animals were fed with algal culture (*Synachocystis salina* @ 5×10^3 cells per ml) daily for 30 min. in raw sea water. On 4th, 8th, 12th and 16th day sufficient animals (16 animals) were removed to study the rate of accumulation of copper. Metal content of the tissues was estimated using Atomic Absorption Spectrometry following method of Armannsson (1979).

Perna indica used to study accumulation of copper nitrate and copper chloride were utilised for oxygen consumption and filtration experiments at the end of accumulation of the metal. Test organisms exposed to copper for 4, 8, 12 and

16 days were placed in 1000 ml conical flask (for oxygen uptake study) and 500 ml beaker (for filtration study) with test solution. 4 animals were introduced in each conical flask and beaker. Gas exchange from the atmosphere was avoided by sealing flask with inert liquid paraffin. The duration of the experiments was 5 to 6 h. The rate of oxygen uptake and filtration of batches of four animals were estimated after 4, 6, 12 and 16 days of bioaccumulation. The frequency of reading was every one hour. Winkler's method and Dye clearance techniques were followed to estimate the dissolved oxygen and filtration respectively. Dye clearance technique involves addition of a known concentration of neutral dye (Neutral red 2.0 ppm) to the test solution and allowing the animals to clear the dye. The quantity filtered was calculated after finding out the reduction in the dye with the help of a spectrophotometer. Filtration rate was estimated using the equation of Abel (1976). The oxygen consumption is expressed as μg 0_2/h/mg (dry weight). The filtration rate is expressed as quantity of sea water in ml filtered by one mussel in one hour.

For studying the rate of depuration of copper, animals exposed to copper nitrate and chloride for 16 days were removed every 4^{th} day and maintained in raw sea water for 7 days. The raw sea water was replenished every 24 h. Metal content of the tissues was estimated following method of Armannson (1979).

Results

Copper irrespective of the two salt forms employed, showed a time bound increase in the rate of accumulation. Thus, the quantity of copper nitrate recorded was 18.74 ppm after 4 days and reached 28.36 ppm after 16 days (figure 1). Similarly, copper chloride recorded after 4 and 16 days was 21.24 ppm and 29.03 respectively. The background (control) concentration of copper in seawater was 0.087 ppb and that in *Perna indica* was 6.51 $\mu g \cdot g^{-1}$ (dry weight). Analysis of variance of the data shows that between treatment combinations the variation noticed, in the rate of uptake of copper, between salts were significantly different (table I).

Table I: *Perna indica* accumulation : Analysis of variance for changes in copper concentration in the whole tissue, when the animals were exposed to 0.5 ppb copper nitrate and copper chloride from 4 to 16 days

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between replicates	3	18.1465	6.0488	2.68 (NS)
2. Between treatment combinations	7	370.3770	52.9110	25.05**
a. Between salts	1	33.1074	33.1074	15.67**
b. Between periods	3	332.1130	110.7043	52.40**
c. Interaction	3	5.1562	1.7187	0.81 (NS)
3. Error	21	44.3652	2.1126	
4. Total	31	432.8887		

NS : Not significant
** : Significant at 1% level

Animals exposed to copper nitrate consumed lesser quantities of oxygen although the load in the whole tissue was low (figures 1 and 2). On the contrary, the animals exposed to copper chloride consumed more oxygen and the whole tissue had relatively large quantities of copper (figures 1 and 2). In the case of those animals exposed to copper chloride, there was non significant difference in the oxygen consumption between periods, whereas significant difference between periods was noticed when the animals were exposed to copper nitrate (table II). The apparent variations noticed in the oxygen uptake between animals exposed to the two salts was found to be insignificant statistically.

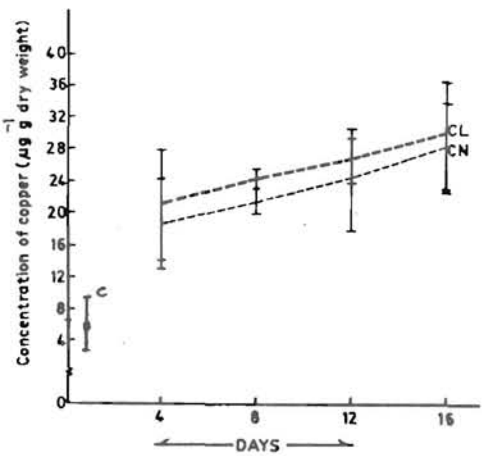

Figure 1: *Perna indica* accumulation: Copper concentration in the whole tissue, when the animals were exposed to 0.5 ppb of copper nitrate (CN) and copper chloride (CL) for period ranging from 4 to 16 days, along with standard deviation

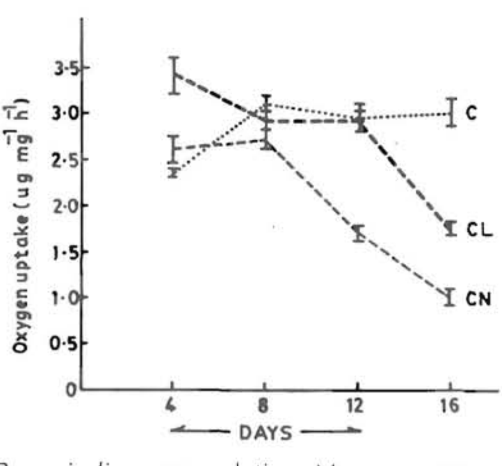

Figure 2: *Perna indica* accumulation: Mean oxygen consumption, when the animals were exposed to 0.5 ppb of copper nitrate (CN) and copper chloride (CL) for period ranging from 4 to 16 days, along with standard deviation

Table II: *Perna indica* accumulation: Analysis of variance for changes in oxygen consumption, when the animals were exposed to 0.5 ppb copper nitrate and copper chloride from 4 to 16 days

A. Copper nitrate

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between periods	4	8.6309	2.1577	7.80*
2. Between replicates	3	1.9387	0.6462	2.33 (NS)
3. Error	12	3.3161	0.2763	
4. Total	19	13.8857		

B. Copper chloride

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between periods	4	5.8882	1.4720	1.70 (NS)
2. Between replicates	3	0.8896	0.2965	0.34 (NS)
3. Error	12	10.3677	0.8639	
4. Total	19	17.1455		

NS : Not significant
* : Significant at 5% level

The rate of filtration decreased as a function of increase in time in both the cases (figure 3). The rate of filtration was significantly different between periods in both the cases (table III). In both the cases, the decrease in filtration rate as time progressed was significantly different.

The rate of depuration of copper was more or less comparable irrespective of the previous accumulation history of the animal. After 7 days of depuration, 4 days animals pre-exposed to copper nitrate gave out 5.17 ppm and those pre-exposed to 16 days 7.50 ppm (figure 4). Similarly, in the case of copper chloride the corresponding values ranged between 5.53 and 7.18 ppm.

Oxygen consumption rate of *Perna indica* during the depuration process was assessed to find out the trend in energy expenditure. Those animals retained for shorter duration i.e., 4 to 8 days in both copper nitrate and copper chloride were found to consume more oxygen after 7 days of recovery (figure 5), whereas those exposed to 12 and 16 days were found to consume less oxygen. There was no significant difference in the oxygen uptake of the test individuals pre-exposed to copper nitrate and chloride for periods ranging from 4 to 16 days after a recovery period of 7 days.

Clear cut variations were noticed in the rate of filtration. Animals pre-exposed to copper nitrate and copper chloride for 4 days, filtered more water after they were maintained in raw sea water for 7 days. The same trend was recorded

Table III: *Perna indica* accumulation: Analysis of variance for changes in filtration rate, when the animals were exposed to 0.5 ppb copper nitrate and copper chloride from 4 to 16 days

A. Copper nitrate

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between periods	4	462.0600	115.5150	7.10*
2. Between replicates	3	83.1659	29.0553	1.78 (NS)
3. Error	12	195.1560	16.2630	
4. Total	19	744.3819		

B. Copper chloride

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between periods	4	742.5040	185.6260	8.03*
2. Between replicates	3	56.5761	18.8587	0.81 (NS)
3. Error	12	277.1040	23.0920	
4. Total	19	1076.1841		

NS : Not significant
* : *Significant at 5% level*

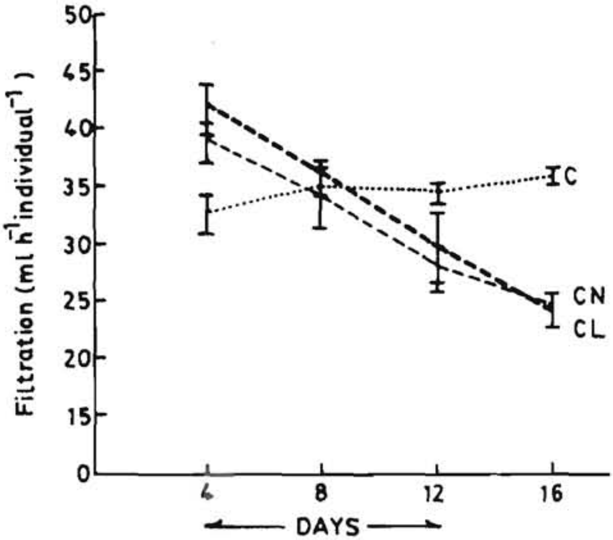

Figure 3: *Perna indica* accumulation: Mean rate of filtration, when the animals were exposed to 0.5 ppb of copper nitrate (CN) and copper chloride (CL) for period ranging from 4 to 16 days, along with standard deviation

Table IV: *Perna indica* accumulation: Analysis of variance for changes in oxygen consumption, when the animals were exposed to raw sea water for 7 days, after being maintained in 0.5 ppb copper nitrate and copper chloride from 4 to 16 days

A. Copper nitrate

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between periods	4	4.8382	1.2095	1.75 (NS)
2. Between replicates	3	2.1705	0.7235	1.04 (NS)
3. Error	12	8.2920	0.6910	
4. Total	19	15.3007		

B. Copper chloride

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between periods	4	4.1000	1.0250	2.30 (NS)
2. Between replicates	3	2.1376	0.7125	1.60 (NS)
3. Error	12	5.3445	0.4453	
4. Total	19	11.5821		

NS : Not significant

Table V: *Perna indica* accumulation: Analysis of variance for changes in filtration rate, when the animals were exposed to raw sea water for 7 days, after being maintained in 0.5 ppb copper nitrate and copper chloride from 4 to 16 days

A. Copper nitrate

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between periods	4	1228.4640	307.1160	12.44**
2. Between replicates	3	112.4667	37.4887	1.51 (NS)
3. Error	12	296.2440	24.6870	
4. Total	19	1637.1747		

B. Copper chloride

Source of variation	DF	SSQ	MSSQ	F. ratio
1. Between periods	4	1803.4080	450.8520	23.91**
2. Between replicates	3	47.9103	15.9701	0.84 (NS)
3. Error	12	226.2576	18.8548	
4. Total	19	2077.5759		

NS : Not significant
** : Significant at 1% level

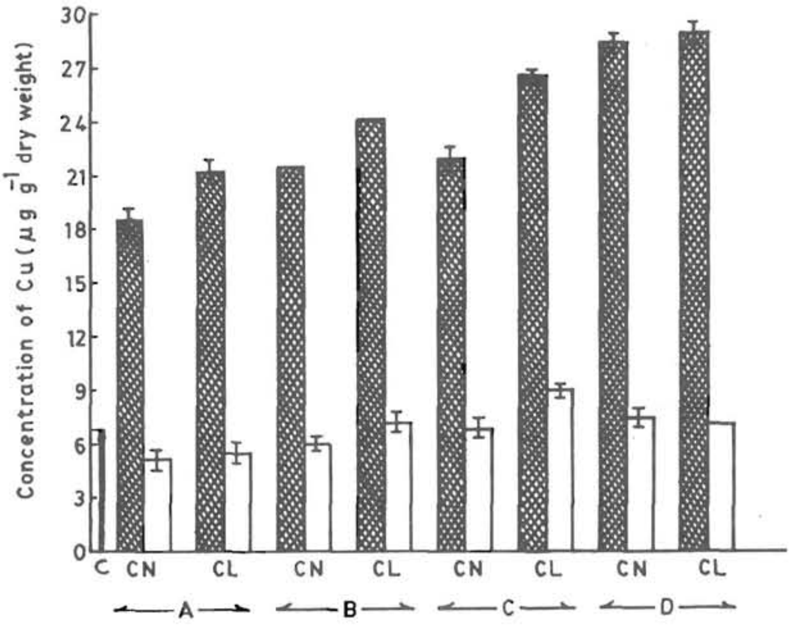

Figure 4: *Perna indica* accumulation Quantities of copper accumulation in the whole tissue (hatched histogram) and quantities of copper depurated in 7 days (open histogram)

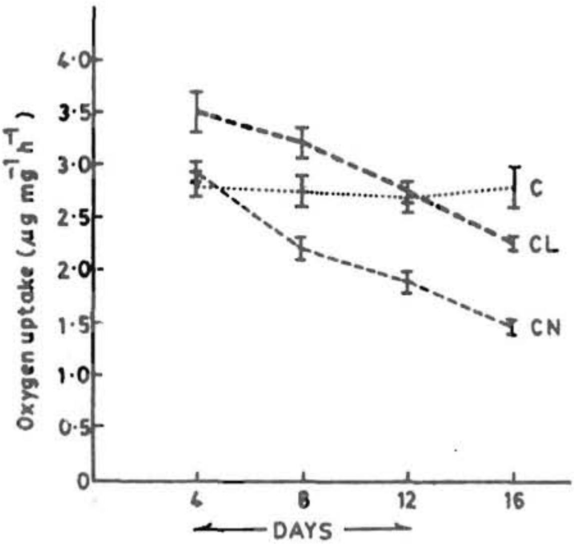

Figure 5: *Perna indica* depuration : Mean oxygen consumption, when the animals were exposed to raw sea water for 7 days, after being maintained in 0.5 ppb of copper nitrate (CN) and copper chloride (CL) for period ranging from 4 to 16 days

by those animals pre-exposed to copper chloride. Both in the case of copper nitrate and copper chloride 16 days pre-exposure and subsequent 7 days maintenance in clean sea water did not result in increase in the rate of filtration (figure 6). Between periods, the rate of filtration by those animals exposed to copper chloride were significant (figure 6). Further, the rate of filtration of those animals pre-exposed to 4 days were significantly different from those pre-exposed to 8, 12 and 16 days and those of control. The same trend was maintained by those animals exposed to copper nitrate also.

Discussion

Heavy metals in trace quantities have been normal constituents of the aquatic environment since the beginning of biological time. It has been proved that the chemical state, as well as the kinetics of transformation between different species of chemical salts dissolved in water influence the rate of bioaccumulation of heavy metals (Mantoura *et al.*, 1978; Allen *et al.*, 1980; Nurnberg, 1983). In another series of experiments, Coombs (1977), Coombs and George (1978) and George and Coombs (1977) proved that the shellfish living in estuaries can be affected by the salt form of metals with reference to uptake. In the present study, the rate of accumulation of copper, when supplied in nitrate and chloride form in very low quantities, 0.5 ppb, showed variations. The level of copper in sea water was 0.087 ppb. The rate of uptake of copper in chloride form was higher than that from nitrate by *Perna indica*. Discussing on salt dependent variations in uptake, Martincic *et al.*, (1984) have suggested various points

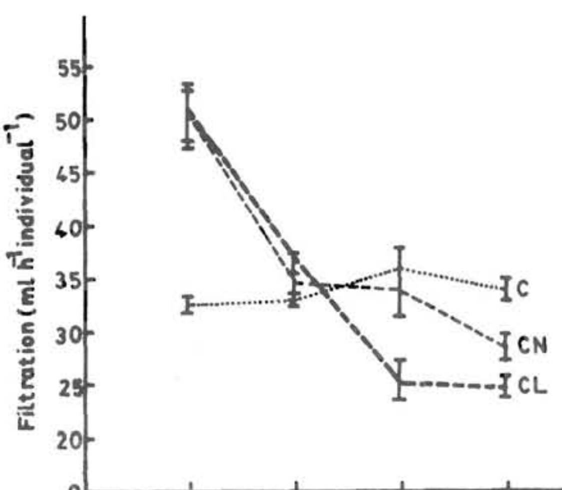

Figure 6: *Perna indica* depuration: Mean rate of filtration, when the animals were exposed to raw seawater for 7 days, after being maintained in 0.5 ppb of copper nitrate (CN) and copper chloride (CL) for period ranging from 4 to 16 days

which influence the differences. They are, the chemical species in which the trace metals exist and variations in metal species depending on the ligand type which might in turn, may or may not favour uptake. Discussing on increased uptake of copper in the oyster, Martincic *et al. (loc.cit.)* suggested that between different species of copper available, oyster pick out the larger part.

The results on depuration show that time limit given for depuration namely, 7 days, did not bring about any clear cut differences in the depuration rate. Irrespective of the previous accumulation history, the quantity thrown out was always more when the body burden was high. This indicates that irrespective of the salt forms of metals, the rate of depuration was controlled by the quantity of copper rather than salt forms supplied.

Rate of oxygen uptake is a useful index to assess the toxicant stress in animals as this indicates the energy expenditure to meet the demand of an environmental alteration (Thurberg *et al.*, 1975). The fact that the rate of oxygen consumption reduced, irrespective of internal load when the animals were exposed for a longer duration show that prolonged exposure distinctly reduces oxygen consumption indicating increased stress. Further, regaining normalcy evidenced by increased rate of oxygen consumption after a depurative process, was recorded only in the case of those animals which had shorter accumulate phase. This indicates that irrespective of the load, exposure for a longer duration tampers with the mechanism of the organs involved in respiratory process, which could be either at the cellular level or tissue level and possibly behavioural level also. *Perna indica* has only limited capacity to regulate oxygen consumption (Hawkins *et al.*, 1986). Discussing the pattern of oxygen consumption in *Perna indica*, Hawkins *et al.* (*loc. cit*) found that the reduced oxygen uptake during the stress period indicate metabolic inhibition. The present finding that prolonged exposure even to lower concentrations of copper reduced oxygen consumption rates even after the animals have been given chance to depurate part of the accumulated metal indicate that the animals are incapacitated to perform normal respiratory action. Among the salts employed, copper nitrate was more toxic than copper chloride.

Filtration rate has a direct relationship with the body burden of heavy metals (Abel, 1976). The present finding show that when the quantity of copper in the tissues was more, the rate of filtration was less. It may be assumed that internal toxicity caused by heavy metals, distinctly affect the filtration rate. The present study indicate that *Perna indica* is more sensitive to the presence of toxicants. The decrease in the quantity of water filtered and increase in oxygen consumption show that the animals probably retain the water that enter the pallial cavity for a longer duration. Repeated circulation of water within the pallial cavity and water bathing the gills which has considerable coating of mucus would have resulted in reducing the dye concentration. Stepping down of particle number below a threshold limit would have eventually resulted in the cessation of filtration and the animals would have used only the dissolved oxygen in water. How far the behavioural responses of *Perna indica,* in influencing, filtration activity at very low sublethal concentrations of heavy metals is unknown. A conspicuous feature of the results obtained in the case of those animals exposed to copper nitrate and copper chloride even after depuration was that the rate of filtration did not increase in the case of those animals which had maximum duration of exposure to toxicants. On the other hand, exposure to 0.5 ppb of copper, either in nitrate or chloride, enhanced filtration rate in the case of those animals exposed for a shorter duration. It may be added here that the body burden of animals exposed for longer duration even after depuration was high.

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